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HIGH FREQUENCY MUSCLES USED IN SOUND PRODUCTION BY A KATYDID. II. ULTRASTRUCTURE OF THE SINGING MUSCLES[1]

HUGH Y. ELDER

Institute of Physiology, The University of Glasgow, Glasgow, W.2., Scotland and Marine Biological Laboratory, Woods Hole, Massachusetts 02543

Males of the tetigoniid *Neoconocephalus robustus* stridulate by rubbing the edges of the forewings together to produce an apparently continuous and high pitched note. The accompanying paper by Josephson and Halverson (1971) has shown that one sound pulse is produced each wing cycle and that the frequency is 145–212 per second. While wing stroke frequencies even much in excess of this have been recorded from insects with asynchronous flight muscles (Smith, 1965a), frequencies in excess of 100 Hz are uncommon in the lower insect orders (Sotavalta, 1947).

The present study was undertaken therefore because it was of considerable interest to determine whether the flight muscles were of the synchronous or of the asynchronous type. The latter type are present only in the orders Coleoptera, Diptera, Hymenoptera, Hemiptera and probably the Thysanoptera (Pringle, 1967). Thus if the muscles were of the asynchronous type it would be a new finding for the Orthoptera and if they were of the synchronous type they would be the fastest described synchronous insect flight muscles and amongst the fastest synchronous muscles in the animal kingdom.

The ultrastructural differences between synchronous and asynchronous (fibrillar) muscles are now well recognized (Smith, 1965b, 1966). One of the most striking features of fibrillar muscle fibers is the almost complete absence of the sarcoplasmic reticulum, which contrasts strongly with the findings in fast neurogenic muscles, from both vertebrates and invertebrates (Fawcett and Revel, 1961; Revel, 1962; Fahrenbach, 1963; Reger and Cooper, 1967), in which the sarcoplasmic reticulum is very well developed, culminating in the extreme condition of a fast acting lobster muscle in which the sarcoplasmic reticulum occupies 75% of the total muscle volume (Rosenbluth, 1969). The structural features therefore of the stridulating mesothoracic muscles of *N. robustus* are examined in relation to other fast acting muscles in an attempt to evaluate those structural features which enable the muscle to function at such high frequencies. They are compared with the flight muscles of the metathoracic segment, which do not participate in stridulation, and with the flight muscles of male *N. ensiger,* a species which overlaps in range with *N. robustus* but stridulates with a frequency of only 10–15 Hz (Heath and Josephson, 1970).

Material and Methods

Males of *Neoconocephalus robustus* and *N. ensiger* were collected from salt marshes in the vicinity of Woods Hole, Massachusetts and kept in outdoor cages until required, as described in Josephson and Halverson (1971). The head and

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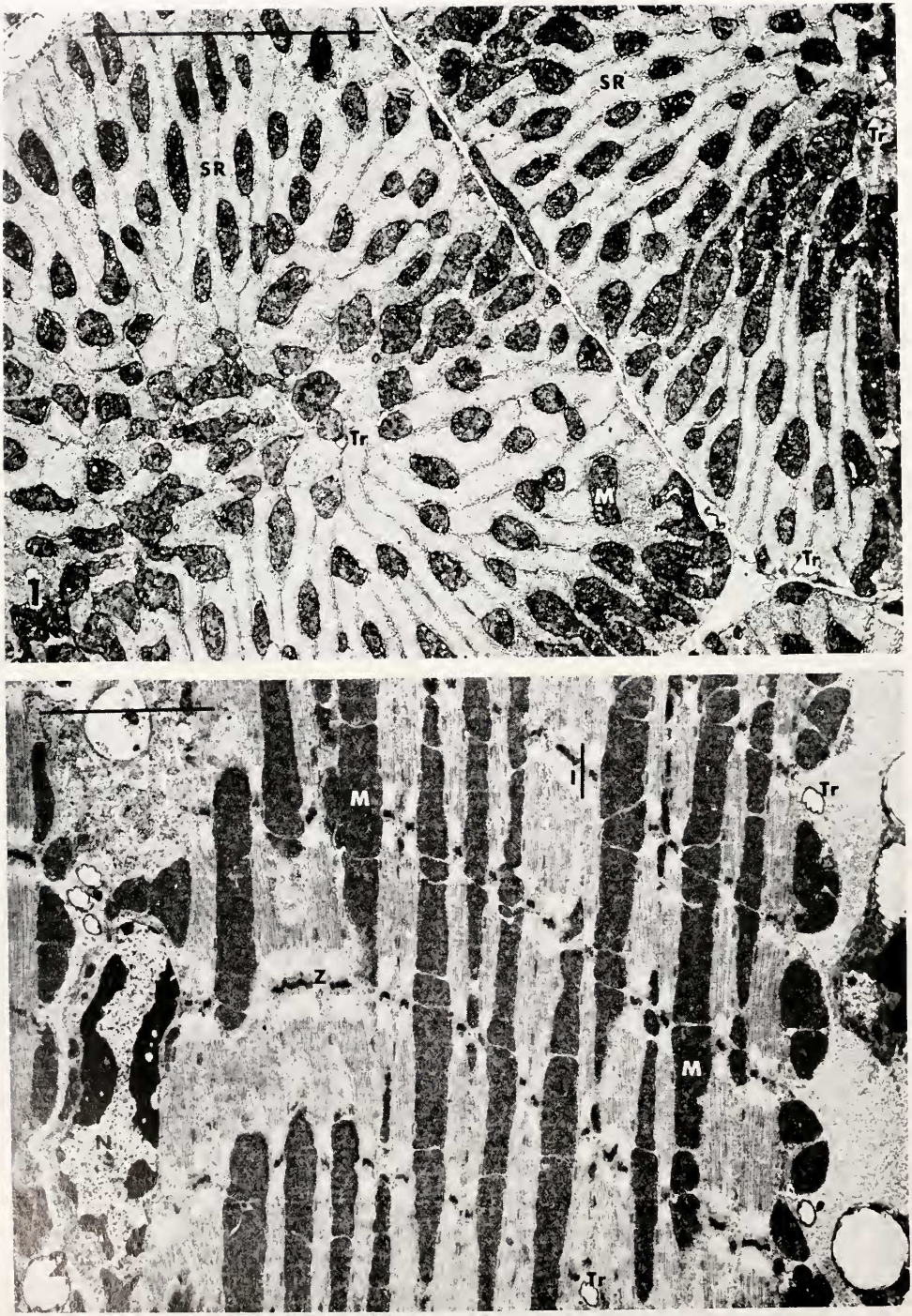
abdomen were removed and the segment of gut pulled out from the thorax. Fixative was either pipetted through the central cavity of the thorax and the whole thorax immersed in fixative or the terga and sterna of the thoracic segments were cut along the mid-line and the halves were immersed in fixative. By either method the attachments of all the muscles to the exoskeleton remained intact. The fixative employed was 3% glutaraldehyde in 0.1 M Millonig phosphate buffer with 3% sucrose at pH 7.4 for 2 hrs at 4° C. The tissues were washed and stored for transport in 5% sucrose in the phosphate buffer at pH 7.4 and 4° C. On the day following fixation the tissues were transported by air in insulated containers to the United Kingdom. On arrival they were post fixed in 1% OsO_4 in 0.1 M phosphate buffer at pH 7.4 and araldite embedded. Samples from the edges of the muscle blocks only were taken for embedding. Duplicate specimens were later taken from insects flown live to the United Kingdom from Woods Hole. Sections were stained with uranyl acetate and lead citrate and examined in an AEI EM6B electron microscope.

For stereometric analysis of the quantity of sacroplasmic reticulum present and the volume of the fibers occupied by mitochondria the combined point count and intersect incidence method of Freere and Weibel (1967) was used. The formulae employed were those for volume estimation (mitochondria and sarcoplasmic reticulum) and surface density (sarcoplasmic reticulum) and the data were obtained by superimposing a cellulose acetate grid with 84 lines and 168 points on electron micrographs of suitable magnification.

Description of fibers

Each of the muscle groups of the stridulating (mesothoracic) flight muscles of *N. robustus,* direct (basalar and subalar), indirect (tergosternal, tergocoxal 1 and 2, pleurotergal and oblique tergal) and median dorsal longitudinal synergists (see Josephson and Halverson, 1971) consists of small fibers of only 10–25 μm in diameter which are arranged around the large central tracheae. Each fiber has peripheral nuclei and the ribbon-like fibrils are radially arranged. Such "radial lamellar" packing with peripheral nuclei is typical of the flight muscles of the Orthoptera (Tiegs, 1955; Pringle, 1965). The metathoracic fibers and meso- and metathoracic fibers of *N. ensiger* are also of this type.

The fibers are ovoid or polygonal in cross section and the strap-like myofibrils are arranged in radial fashion interspersed with numerous large mitochondria (Fig. 1). While the arrangement lacks the almost geometrical precision of some other described flight muscles of the radial lamellar type (Smith, 1962), the form is extremely compact. The mitochondria are subcylindrical in shape, ovoid in cross section and with a rectangular outline when sectioned longitudinally. They are often found packed end to end in rows between fibrils (Fig. 2). Due to the large number of tightly packed cristae the mitochondria are very electron dense. The cristae have a characteristic appearance, often being found tightly packed in sectors of concentrically packed, curved cristae and have the dense appearance and ill-defined cristal detail now recognized as typical of primary fixation with glutaraldehyde (*e.g.*, Ashhurst, 1967). Two configurations were frequently found, normal (Figs. 2, 3, 8) and vesiculated (Figs. 5, 7). In the latter many of the cristae in each mitochondrion of particular fibers had a dilated appearance and it is not clear



FIGURES 1–2.

whether these mitochondrial forms represent configurational changes due to different configurational states (Hackenbrock, 1968) or are fixation artifact such as Stoner and Sirak (1969) have described.

Using stereometric methods (Freere and Weibel, 1967) the mitochondrial volume in the mesothoracic flight muscles of male *N. robustus* was found to be 44% of the total fiber volume (Josephson and Elder, 1968). Although the mitochondria in the metathoracic fibers are of the same appearance as those described above they are significantly fewer in number (Fig. 3).

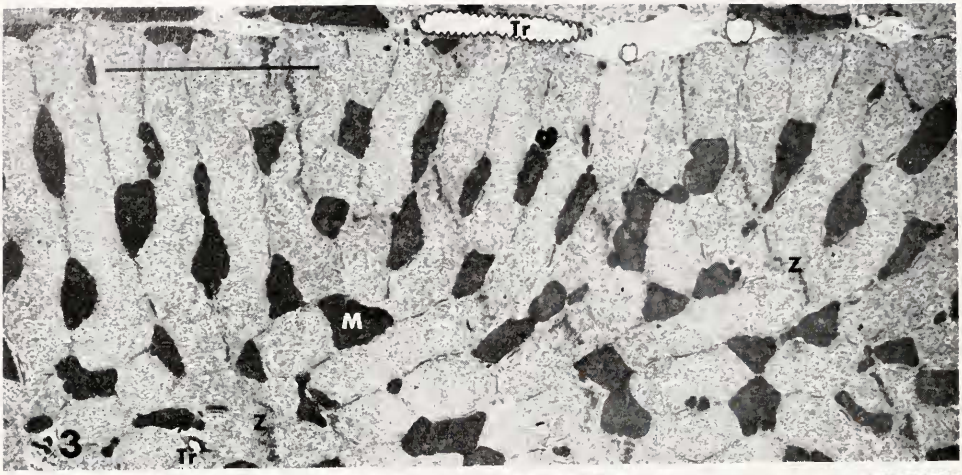
The sarcomeres are short (approximately 3 μ) with narrow I-bands. The sarcomeres of the median dorsal longitudinal muscles are significantly longer than those of the other direct and indirect flight muscles (approx. 4.2 μ). The margins of the A-bands are rather ill-defined and M-lines are absent. The thick filaments of the A-band are approximately 2.4 μ in length (3.3 μ in the median dorsal longitudinal muscles). In all fibers the myosin filaments are approximately 16 nm in diameter with a center to center spacing of approximately 48 nm. The thin filament margins in the A-band are also not in perfect register and thus in longitudinal sections an H-band is difficult to distinguish. In transverse sections of relaxed muscle the presence of the H band is recognizable by the absence of thin filaments (Fig. 5). Transverse sections show the thick filaments disposed in hexagonal array and each thick filament is surrounded by an orbit of six thin filaments. Each thin filament is located equidistant between adjacent pairs of thick filaments, as in many other insect flight muscles (Spiro and Hagopian, 1967), which gives a thin to thick ratio of 3:1 (Smith, 1966). In contracted fibers orbits of twelve thin filaments around each thick filament are found in the central regions of the A-bands due to "double overlap" of the thin filaments (Fig. 4).

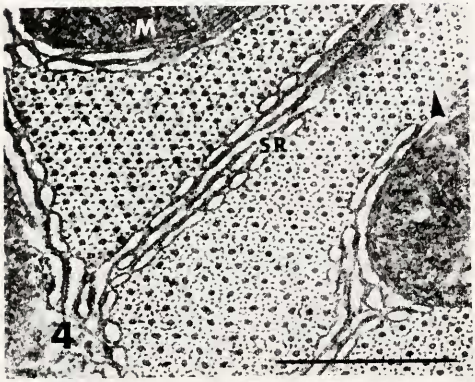
Glycogen granules are found mainly near the Z-line regions between fibrils but they also occur in rows between thin filaments in the I-band regions within fibrils (Figs. 11, 12).

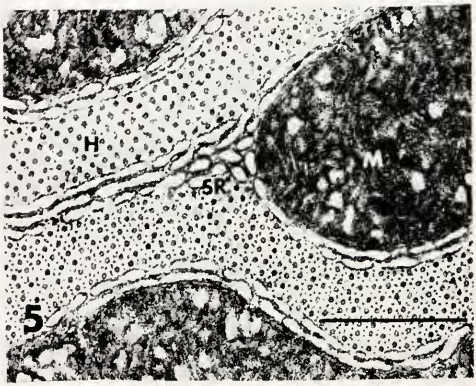
The sarcoplasmic reticulum (SR) is very well developed, particularly in the mesothoracic flight muscles of *N. robustus* (Figs. 4–6). In sections which lie for some distance in interfibrillar spaces the form of the SR is seen to be that of a continuous network of interconnected tubules forming perforate curtains which completely surround each fibril (Figs. 6, 9, 10). In many locations overlapping has occurred so that between fibrils there may be as many as four or more layers of SR tubules (Figs. 4–6). A layer of SR is found between the myofibrils and the mitochondria so that all fibrils are completely surrounded by at least one thickness of SR membranes. The stereometric estimation shows that the SR occupies

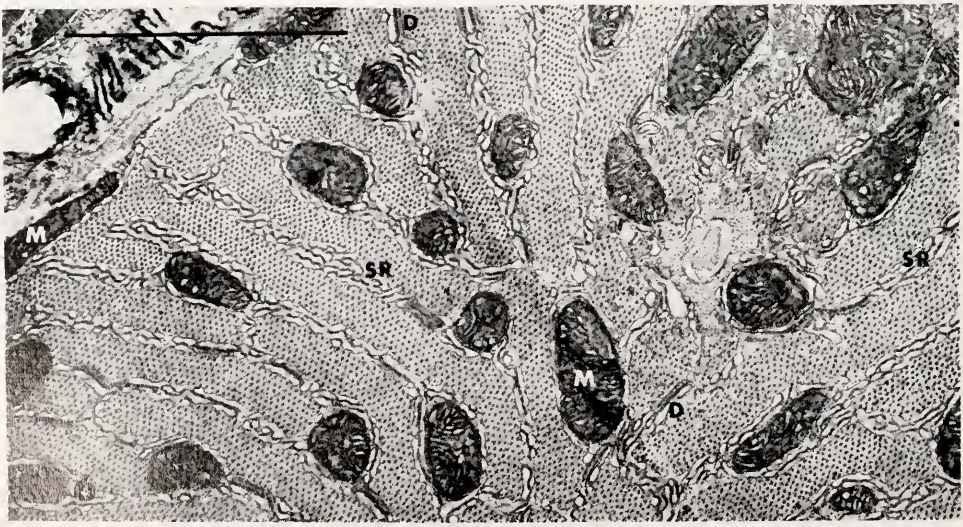
FIGURE 1. *N. robustus,* transverse section of the mesothoracic median dorsal longitudinal muscle shows the form of the radially arranged narrow fibrils and the numerous, large mitochondria. Well developed sarcoplasmic reticulum surrounds the fibrils and tracheae running longitudinally in the center of the fiber are seen. Abbreviations are: D—Dyad, G—Glycogen granules, H—H band, I—I band, M—Mitochondria, N—Nucleus, SR—Sarcoplasmic reticulum, T—T system cleft, Tr—Tracheole, Z—Z line; scale line = 5 μ.

FIGURE 2. *N. robustus,* longitudinal section of the mesothoracic tergocoxal 1 muscle. The nodular profile of a fiber, caused by the peripheral location of mitochondria opposite the A-bands in many sarcomeres is seen. Most of the mitochondria have a rectangular profile and pack in rows between the fibrils. Numerous tracheae and the peripheral nuclear location are also seen. In this extended region of the fiber the I-band is prominent; abbreviations as in Figure 1, scale line = 5 μ.









FIGURES 3–6.

approximately 19% of the total fiber volume and has a surface area of approximately 14 μ^2 per μ^3 of fiber volume. Stereometric analyses of the fibers from *N. ensiger* or of the metathoracic fibers of *N. robustus* have not been made but it can be seen that the development of the SR of the mesothoracic fibers of *N. ensiger* approaches that in *N. robustus* (Fig. 7) while the metathoracic flight muscles of both species have the same pattern of SR organization but clearly less well developed than in the mesothoracic segments (Figs. 3, 8).

T-system clefts (30 to 40 nm by 120 to 360 nm) invaginate at the level of overlap of the thick and thin filaments (Figs. 8, 9), *i.e.*, two invaginations per sarcomere length. At regular intervals along each sarcomere of each fibril dyads are formed by the close association of a T-tubule and an overlying cysternal plaque of the SR. These are located in the A-band at the regions of overlap of thick and thin filaments (Fig. 9). The T-system invaginations are not of uniform dimensions along their length. Where dyads are formed the T-system clefts expand to form periodic plaques up to 600 nm across (Fig. 10). Electron dense granular material is frequently seen in the T-tube lumina of the dyads. The spacing of these granules appears to be fairly regular at about 38 nm (Fig. 11). Since the form of the T-system is that of a flattened cleft rather than that of a tubule with a cylindrical cross section, invaginations are more readily found in transverse sections (Fig. 8) than in longitudinal sections (Fig. 9). In the mesothoracic fibers of both *N. robustus* and *N. ensiger* the presence of peripherally located mitochondria gives these fibers a nodular profile in longitudinal section (Fig. 2) which frequently causes the T-system invaginations to be displaced longitudinally to such an extent in some locations that the invaginations are initially found to run longitudinally to reach the location of dyad formation at the region of thick and thin filament overlap (Fig. 12).

Periodic large diameter invaginations of the sarcolemma, at the level of the Z-lines, allow tracheoles to penetrate the full radius of the muscle fibers and one or more tracheoles are frequently found running longitudinally at the center of each fiber (Figs. 1, 3). At the periphery of the fibers the Z-lines attach to the plasma membrane by hemi-desmosomes. Seen in longitudinal section the Z-lines are rather broad, ill-defined, electron dense areas (Figs. 2, 12). In contracted muscle the

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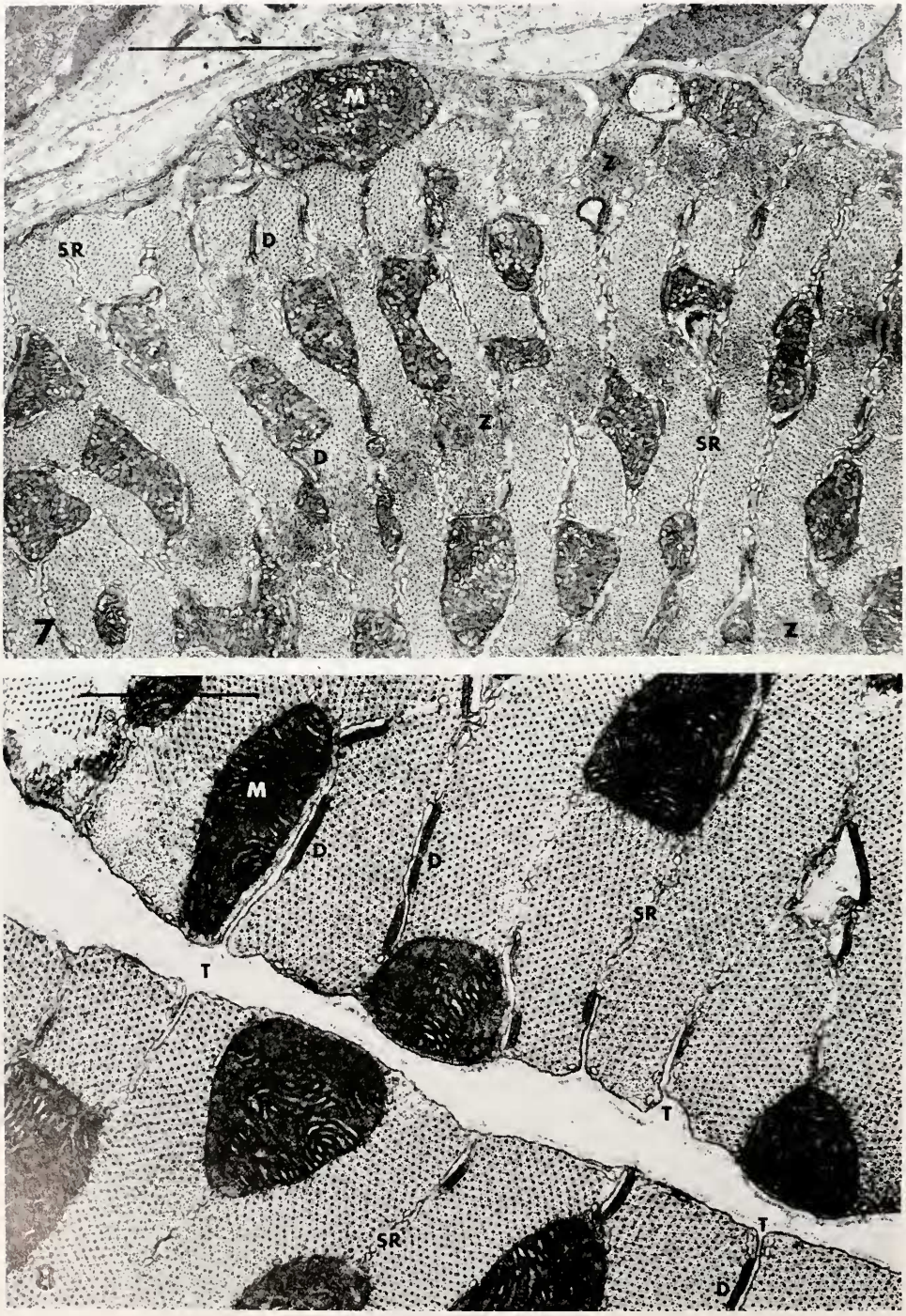
FIGURE 3. *N. robustus,* transverse section of the metathoracic median dorsal longitudinal muscle. The fibril organization is radial lamellar but the fibrils are wider, the mitochondria are less numerous and the SR is less well developed than in the singing muscles; abbreviations as in Figure 1, scale line = 5 μ.

FIGURE 4. *N. robustus,* transverse section of the mesothoracic first tergocoxal muscle. The normal insect flight muscle filament packing pattern with six thin filaments surrounding each thick filament and with the thin filaments equidistant between adjacent thick filaments is seen at the top of the figure (*e.g.,* arrow). At the bottom of the figure double overlapping of thin filaments giving orbits of up to twelve around each thick filament is apparent. The well developed SR and vesiculated mitochondria are again prominent; abbreviations as in Figure 1, scale line = $\frac{1}{2}$ μ.

FIGURE 5. *N. robustus,* transverse section of the mesothoracic first tergocoxal muscle. The narrow strap-like fibrils are surrounded by layers of SR tubules and the H-band is apparent in this field. The mitochondria have a vesiculated appearance (see text) and the myosin filaments appear to be hollow; abbreviations as in Figure 1, scale line = $\frac{1}{2}$ μ.

FIGURE 6. *N. robustus,* transverse section of the mesothoracic median dorsal longitudinal muscle shows the extensive development of the SR tubules which ensheath the ribbon-like fibrils. In most locations there are at least two layers of SR and in some, up to five; abbreviations as in Figure 1, scale line = 2 μ.



FIGURES 7–8.

thick filaments penetrate some way into the electron dense Z-line material and while no myosin filaments have been seen completely penetrating a Z-line and nothing approaching the supercontraction described by Osborne (1967) and others has been observed, the situation in the katydid flight muscles appears to be very similar to that described by Hagopian (1970) in chick muscle.

Discussion

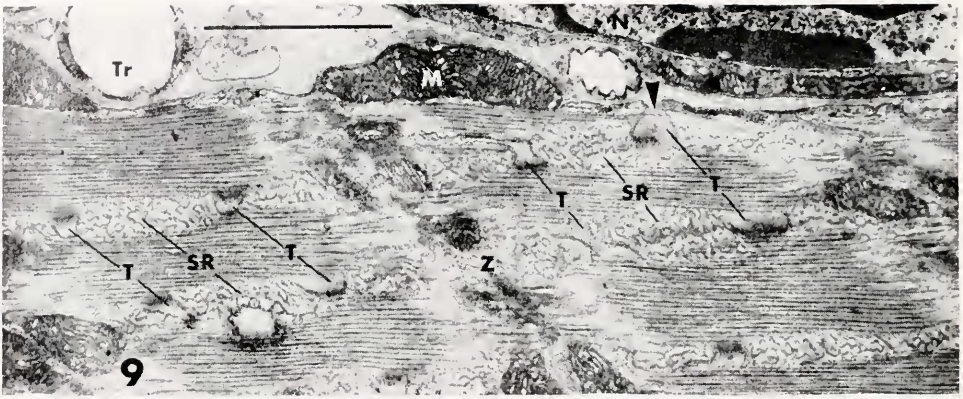
The evidence of Heath and Josephson (1970) indicates that the metabolic activity of the mesothoracic flight muscles of *N. robustus* is very high during stridulation. The more metabolically active a tissue is, the greater the number of mitochondria it contains and amongst skeletal muscles it is the tonic muscle more or less continually active in postural activity, and the phasic muscles specialized to produce the fastest speed which have the highest number of mitochondria (Hoyle, 1969). Josephson and Elder (1968) found the high value of 44% of the total fiber volume occupied by mitochondria in the flight muscles used in stridulation by *N. robustus*. The figure is similar to that calculated by Smith (1961) for *Aeshna* flight muscle, another fast acting synchronous flight muscle.

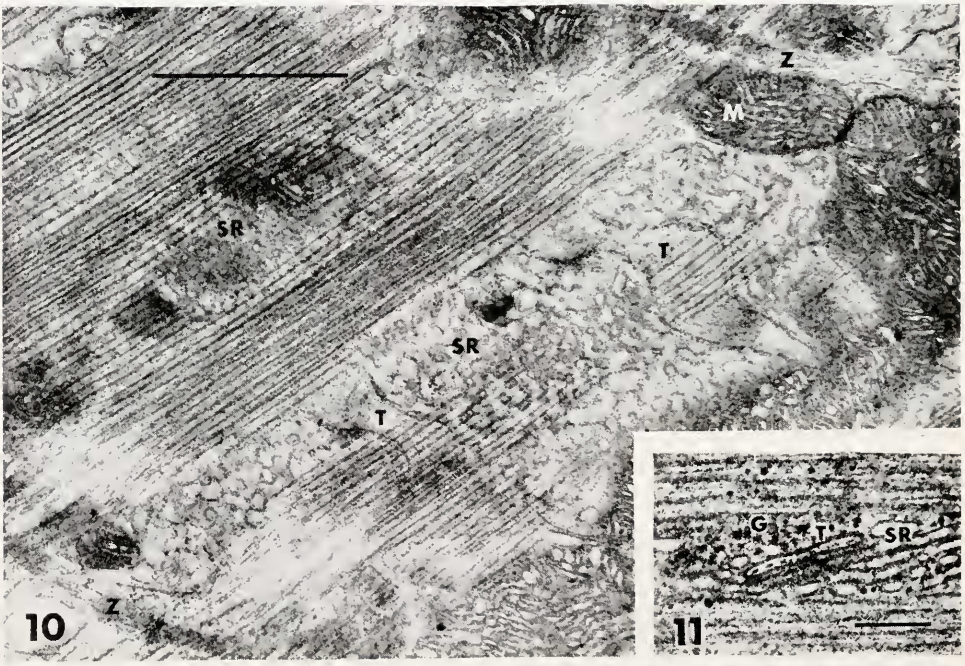
Amongst fish very fast acting muscles with a mechanical response of up to 200 Hz have been described by Fawcett and Revel (1961). Revel (1962) has described a muscle with a similar contraction rate amongst mammals. The ultrastructure of some fast acting crustacean muscles has been described by Farenbach (1963, 1964) and Rosenbluth (1969). Sotavalta (1947) measured the wing stroke frequency of a large number of insects and recorded frequencies up to several hundred cycles per second and even in excess of 1000 Hz. All such very high frequencies have been found hitherto amongst the five insect orders with asynchronous muscles (Pringle, 1967). Following Pringle's (1949) work it was appreciated that these muscles represent a special category of muscle in which the contraction frequency may be far in excess of the rate of efferent impulses. It was shown by Boettiger (1951) that the oscillatory frequency of these muscles depends upon the load and not upon the frequency of neuronal stimulation.

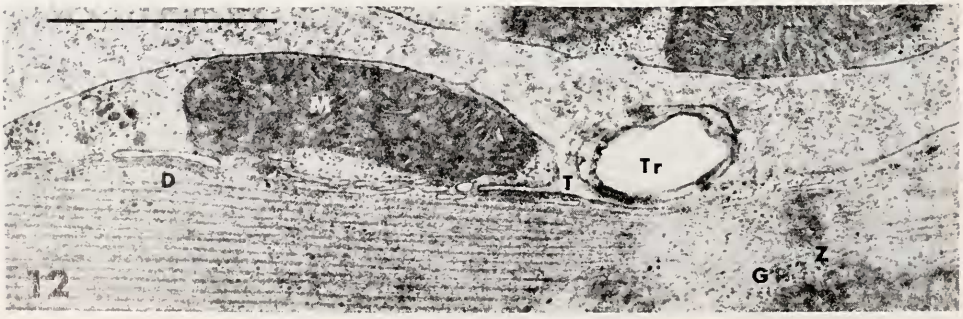
By contrast the synchronous flight muscles found in other insect orders, including the Orthoptera, although providing examples of fast acting muscles, rarely exhibit frequencies above 100 Hz (Sotavolta, 1947), although Josephson and Halverson (1971) have predicted that the very fast wing stroke frequency (280 Hz) found in stridulating *Orocharis gryllodes* (Walker, 1969) will prove to be powered by synchronous muscle. Ultrastructural studies of synchronous muscles from insects with relatively high wing stroke frequencies have been made by Auber (1967), Reger and Cooper (1967) and others. With a mechanical response of 150 to 200 Hz the muscles described in the present study appear to be the fastest synchronous

Figure 7. *N. ensiger*, transverse section of the mesothoracic first tergocoxal muscle. The singing muscles of this species have a very similar appearance to those of *N. robustus*. Mitochondria are numerous and the well developed SR surrounds the narrow fibrils; abbreviations as in Figure 1, scale line = 2 μ.

Figure 8. *N. ensiger*, transverse section of the metathoracic median dorsal longitudinal muscle. In this species also the metathoracic fibers have broader fibrils and a less well developed SR than those of the mesothorax. In this field, from the region of overlap of thick and thin filaments, a number of T-system invaginations are seen to form dyads with elements of the SR containing electron dense material; abbreviations as in Figure 1, scale line = 1 μ.







FIGURES 9–12.

flight muscles so far described and it is of value to compare their structure with that of the asynchronous type and with other known fast acting vertebrate and invertebrate muscles.

The structure of insect fibrillar muscles is well known from the papers of Tiegs (1955), Smith (1961, 1966) Shaffiq (1963, 1964), Ashhurst (1967), and others. The fibers are usually of large diameter (100-200 μ) with well spaced cylindrical fibrils, 1–3 μ in diameter. The transverse tubules are variably placed but frequently at the level of the M-line. The sarcoplasmic reticulum is strikingly reduced and limited to the cisternae of the dyads, Z-line vesicles and a few other irregularly placed tubules. The sarcomeres are short, the I-band is narrow and an M-line, unusual in insect muscle, is present. The tapered ends of the myosin filaments are attached to the Z-band material. Numerous dense and irregularly shaped mitochondria pack much of the space between the fibrils.

In most of these respects the muscle in the present study differs strikingly from the fibrillar muscle characteristics and corresponds to other described synchronous insect flight muscles of the radial lamellar type. Thus the fibers are only some 10–25 μ in diameter with ribbon-like fibrils only 200–400 nm wide arranged in radial fashion. Sarcoplasmic reticular tubules totally surround each fibril. Stereometric estimation shows that the vesicles occupy approximately 19% of the total fiber volume and have a surface area of some 14 μ^2 per μ^3 of fiber. T-tubes invaginate regularly opposite every A/I overlap region. Dyadic associations between the T-tubes and the SR membranes thus occur twice per short sarcomere length (3–4.2 μ) which, combined with the narrow fibril width, means that a very large number of T-tube invaginations are present. Calculation suggests that the incidence would be at least one T-tube invagination per μ^2 of muscle membrane. This compares with an incidence of approximately only 0.6 T-tube invaginations per μ^2 of membrane in frog sartorius muscle. The internal volume and surface area of the T-system will be much greater in frog sartorius, however, since the diameter of the latter is 5–10 times greater than that of the singing flight muscles. But estimated as volume or surface area per unit volume of fiber the quantities in these muscles may be similar, for little branching of the T-tubes was observed amongst the radially oriented, ribbon-shaped fibrils of *Neoconocephalus,* while Peachey and

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FIGURE 9. *N. robustus,* longitudinal section of the mesothoracic median dorsal longitudinal muscle. The invagination of a T-system cleft is contained within the thickness of the section (arrow). The plane of section passes obliquely through the SR ensheathing several fibrils. Two T-system clefts penetrate the fiber at the regions of overlap of thick and thin filaments in each sarcomere; abbreviations as in Figure 1, scale line = 2 μ.

FIGURE 10. *N. robustus,* longitudinal section of the mesothoracic median dorsal longitudinal muscle. The SR and T-system clefts are seen *en face.* Frequent branching of the SR tubules forms a perforate curtain which extends the length of the sarcomere. The T-system clefts are periodically expanded into plaques; abbreviations as in Figure 1, scale line = 1 μ.

FIGURE 11. *N. robustus,* longitudinal section of the mesothoracic first tergocoxal muscle at the region of dyad formation. The T-tubule frequently contains regularly spaced, electron dense granules in its lumen and the cisternal element of the SR contains finely granular, electron dense material; abbreviations as in Figure 1, scale line = 200 nm.

FIGURE 12. *N. robustus,* longitudinal section of the mesothoracic first tergocoxal muscle. Peripherally located mitochondria at the A-band regions appear to cause the longitudinal displacement of the T-system invaginations which therefore run longitudinally in their initial course in order to reach the region of dyad formation in the A-band. A dyad, probably formed in this way, is seen to the left of the figure; abbreviations as in Figure 1, scale line = 1 μ.

Schild (1968) have shown that the quantity of T-system present in frog sartorius muscle is some 30% greater due to branching than that estimated on the basis of incidence of surface invaginations at the Z-lines. While additional "Z-invaginations" (Peachey, 1968) are present primarily to conduct tracheoles into the depth of the fibers, no dyadic associations have been observed between them and elements of the SR such as have been observed in other arthropod fibers (Brandt, Reuben, Girardier and Grundfest, 1965; Cochrane, Elder and Usherwood, in press). No M-line is present and although the I-bands are narrow the thick filaments are not attached to the Z-line. Thus it is clear that these muscles have a structure typical of other radial-lamellar, synchronous muscles (*e.g.*, Smith, 1962, 1966).

Several of the ultrastructural features of the mesothoracic flight muscles of *N. robustus* can be interpreted as adaptations to a fast contraction-relaxation cycle. The very small diameter of these fibers will limit the length of the transverse tubular invaginations and therefore presumably minimize the time taken by the inward spread of excitation. Gonzalez-Serratos (1966), working with frog sartorius muscle, calculated that 8 cm/sec was the speed of inward spread of excitation at 20° C and that the velocity increased with temperature up to that point. Heath and Josephson (1970) have demonstrated that the functional temperature of the stridulating muscles in *N. robustus* is 36° C, some 10° C higher than ambient, and a value of 8 cm/sec for the inward spread of excitation might therefore be low for this insect muscle. However, if the figure of 8 cm/sec is adopted, this phase of the excitation/contraction coupling process in *N. robustus* muscles would take only 0.1 msec in fibers of 8 μ radius. The high incidence of T-tube invaginations in these muscles must represent an adaptation for achieving a rapid and even spread of excitation throughout the fiber. By contrast, the fast acting (100–130 Hz) remotor muscle of the lobster second antenna described by Rosenbluth (1969) forms a syncitium with connective tissue septa making incomplete longitudinal partitions at 50–100 μ intervals. T-tube-like extensions from the septa form dyads with cisternae of the SR at the region of overlap of thick and thin filaments. However, membrane depolarization is accompanied by Ca^{++} influx from the extracellular fluid (Mendelson, 1969), unusual in fast acting muscles, and Rosenbluth (1969) suggests that the entry of Ca^{++} ions from the extracellular fluid may directly trigger contraction or augment the action of Ca^{++} release from the terminal cisternae of the dyads.

Similarly the very narrow fibril width may well be an adaptation to minimize the time necessary to achieve an even distribution of Ca^{++} ions amongst the myofilaments by diffusion from the SR. Winegrad (1968) has shown that in frog skeletal muscle Ca^{++} is released from the SR terminal cisternae of the triad during activation and during relaxation is sequestered by the SR tubules surrounding the fibrils. Although the SR cisternal element of the dyad in synchronous flight muscle is not as well defined as the terminal cisternae of the vertebrate triad a similar mechanism may operate in these arthropod muscles. Thus although the length of the diffusion pathway in the singing flight muscles of *N. robustus* is not known exactly it must lie between approximately 1 μ and 100–200 nm. At maximum it would require only 1 msec for Ca^{++} ions to become evenly distributed over 1 μ (Ebashi and Endo, 1968) and a very small fraction of the 1 msec if the shorter distances are applicable since the time required for distribution depends upon the square of the linear dimension. Narrow fibril width (Fawcett and Revel, 1961; Revel, 1962; Smith, 1962) or a functional reduction in the distance between filaments and sarcoplasmic

reticulum (Farenbach, 1963; Auber, 1967) is a normal feature of fast acting synchronous muscle of both vertebrates and arthropods.

The very large amount of SR present is a feature of the fast acting flight muscle and it seems probable that the greatly increased ratio of SR surface area to myofilament number (SR occupies approximately 19% of total fiber volume; filaments occupy approximately 36% of total fiber volume) is an adaptation to decrease the delay between stimulation and the release of that quantity of Ca^{++} ion necessary to initiate the mechanical response. Large quantities of SR are characteristic of other fast acting muscles (with the exception of insect asynchronous flight muscle) and it is well established that there is a relation in many muscles between speed of contraction and amount of SR present (Bendall, 1969; Hess, 1970; Cochrane *et al.*, in press). In the fast acting lobster muscle described by Rosenbluth (1969) the SR occupies three quarters of the total fiber volume. Relaxation is very rapid in these muscles (Mendelson, 1969) and the calculations of Van der Kloot (1969) have shown that the rate of Ca^{++} binding by the SR would be a function of the amount of SR surrounding the myofibrils even to the remarkable quantities present in the lobster remotor muscle. Such SR development greatly exceeds the SR volume estimated for the mesothoracic fibers in *N. robustus*. As Mendelson (1969) has pointed out, however, the large volume in the remotor muscle may be required in order that it can achieve contraction frequencies of 100 Hz at 16–18° C; some 20 C° lower than the muscle temperature in singing *N. robustus* (Heath and Josephson, 1970).

The singing flight muscle fibers of *N. robustus* have a short striation pattern (thick filament length of 2.4 μ and sarcomere length of approximately 3 μ) amongst arthropod muscles and even amongst flight muscles (Spiro and Hagopian, 1967). A short sarcomere length seems an obvious adaptation to fast action, since for a given total fiber shortening, a muscle with short sarcomeres will undergo smaller percentage shortening of the sarcomere than one with long sarcomeres. In terms of the cyclical action of attachment and detachment of myosin bridges between thick and thin filaments, the shorter the sarcomere length, the fewer would need to be the cycles of cross-bridge make and break in a given time to produce a given total fiber contraction. In general, where sarcomere lengths vary, fast acting fibers have short sarcomere lengths (Spiro and Hagopian, 1967; Hoyle, 1969). Also the length by which insect flight muscles shorten is much less, as little as 5% in synchronous flight muscles *in vivo* (Weis-Fogh, 1956), than in most other muscles. This appears also to be the case in katydid flight muscles (R. K. Josephson, University of California, Irvine, personal communication). Further, the rate of rise of the twitch response may be greater in these muscles, as in other synchronous flight muscles, because of a relatively non-complaint series elastic component (Buchthal, Weis-Fogh and Rosenfalck, 1957; Pringle, 1965). Bárány (1967) found a 200 fold difference in ATPase activity amongst a variety of different vertebrate muscles; it would not be surprising to find high enzyme activities in *N. robustus* singing muscles also as a further adaptation to fast action.

The structural features of the singing flight muscles have been discussed above in an attempt to recognize those features which may be of importance in conferring the ability to respond at the very high frequencies found. There remains, however, the enigma of why the stridulating muscles of *N. ensiger*, which sings with the much lower frequency of 10–15 Hz (Heath and Josephson, 1970), are structurally

very similar. Nor do the flight muscles of members of such well described groups as the Odonata (Smith, 1961, 1966) or species of the Lepidoptera with slow wing beat frequencies, such as *Pieris* or *Vanessa* (Auber, 1967; Reger and Cooper, 1967; Smith, 1962) or the locust *Schistocerca* (personal observations) differ strikingly in any of the essential features such as development of the T-system or sarcoplasmic reticulum or dimensions of the fibers and fibrils, despite probably unimportant differences in fiber architecture (tubular fibers in the Odonata and close packed fibers in the Lepidoptera). Yet they too operate, in flight, at a functional frequency approximately an order of magnitude slower than the stridulating flight muscles of *N. robustus*. The explanation may be that, as in *Schistocerca,* although the twitch is of brief duration (Buchthal *et al.,* 1957; Neville and Weis-Fogh, 1963) the wing frequency is limited by the long refractory period of the motor nerves which impose a maximum impulse frequency (Ewer and Ripley, 1963). The evidence of Josephson and Halverson (1971) shows that in singing *N. robustus* the flight muscles are driven at a frequency of 150–200 Hz by a central nervous pacemaker. It seems probable therefore that the much slower stridulation frequency in *N. ensiger* results not from any inability of the muscles to respond faster but from an imposed neural frequency. It may be concluded that in an Orthopteran already endowed with fast twitch muscles the greatest adaptation to high frequency wing movement has not been to increase the contraction/relaxation rate so much as in the provision of the central nervous pacemaker and refractory properties of the motor nerves. *N. robustus* may simply have exploited a potential which may already be present in many insect orders with synchronous flight muscles and the apparent lack of any striking structural difference between the stridulating muscles of the two species may therefore not be so surprising. In either species there is a greater difference between the mesothoracic singing muscles and those of the metathorax, used solely for flight, than there is between the singing muscles of the two species. The full significance of this adaptation to stridulation remains to be clarified.

Summary

1. The forewings of male *Neoconocephalus robustus* are rubbed together at frequencies of 150–200 Hz during singing. The ultrastructural organization of the mesothoracic flight muscles is typical of synchronous flight muscle.

2. The small diameter fibers (10–25 μ) are well supplied with tracheole branches which invaginate at the Z-lines. The radially arranged, ribbon-like fibrils are only 200–400 nm across.

3. Numerous, large mitochondria (44% of total fiber volume) have tightly packed cristae indicative of a high metabolic activity in these muscles. The cristae were observed in two configurations, normal and vesiculated.

4. The well developed sarcoplasmic reticulum occupies 19% of the total fiber volume and has a surface area of 14 μ^2/μ^3 of fiber. It completely invests the fibrils.

5. T-tube invaginations (incidence, 1 per μ^2 fiber surface) form dyads with the sarcoplasmic reticulum at the overlap regions of thick and thin filaments in each sarcomere. The thin: thick filament ratio is 3:1 as in other insect flight muscles. Thick filaments penetrate some distance into the broad Z-lines in contracted specimens.

6. Ultrastructural features of this very fast acting synchronous muscle have been compared with those of other described fast acting muscles and with other katydid flight muscles.

7. The ultrastructure of the singing flight muscles of *N. ensiger* which employs a wing frequency of 10–15 Hz during singing shows no striking differences from the singing flight muscles of *N. robustus;* the synchronous flight muscle is probably pre-adapted to a fast contraction/relaxation cycle and the greatest specialization may lie in the central nervous pacemaker and other neural characteristics.

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